PO-CON1745E

SHIMADZU
Excellence in Science

Highly sensitive and rapid analysis of synthetic dyes in sea food by LC/MS/MS

ASMS 2017 MP 189

Shailendra Rane[1], Ashutosh Shelar[1], Shailesh Damale[1], Rashi Kochhar[1], Purshottam Sutar[1], Deepti Bhandarkar[1], Anant Lohar[1], Ajit Datar[1], Pratap Rasam[1], Jitendra Kelkar[1] and Devika Tupe[2]

1 Shimadzu Analytical (India) Pvt. Ltd., 1 A/B Rushabh Chambers, Makwana Road, Marol, Andheri (E), Mumbai-400059, Maharashtra, India.

2 Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University, Ganesh khind Road, Pune-411007, Maharashtra, India.

Highly sensitive and rapid analysis of synthetic dyes in sea food by LC/MS/MS

Introduction

Synthetic dyes like malachite green, crystal violet are used for wide range of industrial applications. However, they are also used in aquaculture due to their anti-bacterial and anti-fungal properties. They are cheap, very effective and readily available, but due to their toxic effects to humans, these dyes are banned in the EU and US regions with zero tolerance policy. [1] [2]

Here, LC/MS/MS method has been developed for quantitation of malachite green, leucomalachite green, crystal violet and leucocrystal violet from sea food sample using LCMS-8045, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan.

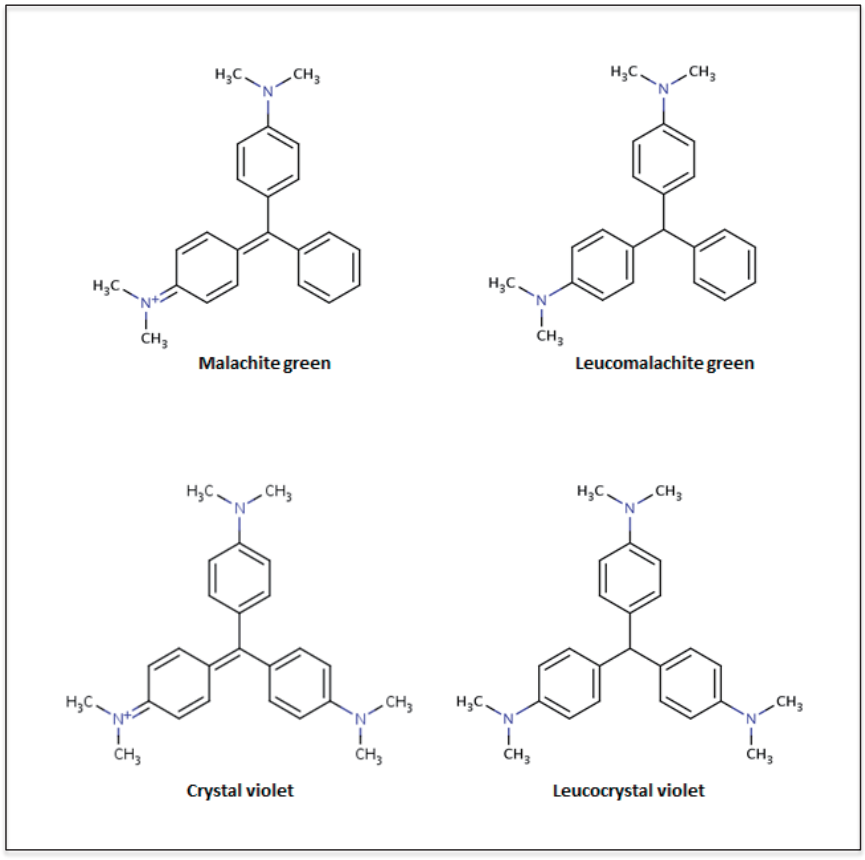

Figure 1. Structure of synthetic dyes

Methods and materials

Sample preparation

Synthetic dye (Figure 1) standards procured from Sigma-Aldrich were used for the analysis. All individual standard stocks were prepared in the acetonitrile. Further mixture of all dyes were prepared in acetonitrile. This stock was serially diluted to prepare calibration levels ranging from 0.05 ppb to 10 ppb in acetonitrile for solvent standard and in matrix for matrix matched standard calibration.

Analysis of these dyes is difficult due to their instability. These synthetic dyes readily undergo oxidation-reduction reaction hence to stabilize them ascorbic acid as an anti-oxidant was added prior to the sample extraction. [3]

For sample analysis, commercially available shrimp sample was purchased and used for analysis. Sample was crushed and transferred to 15 mL centrifuge tube to which 10 mL of acidified acetonitrile and 1 mL of 1M solution of ascorbic acid was added. It was kept on mechanical shaker for 15 min for proper extraction of dyes. Sample were centrifuged for 10 min at 3000 rpm at 4 °C. Then the supernatant layer was transferred to 50 mL tube containing 20 mL Mcllvaine's buffer. Phenomenex Strata SCX SPE cartridges were used for sample clean up. Methanol containing 1% triethylamine and 0.5% formic acid was used as an elution solvent for SPE. After clean up, sample were injected on LCMS-8045.

Figure 2. Nexera with LCMS-8045

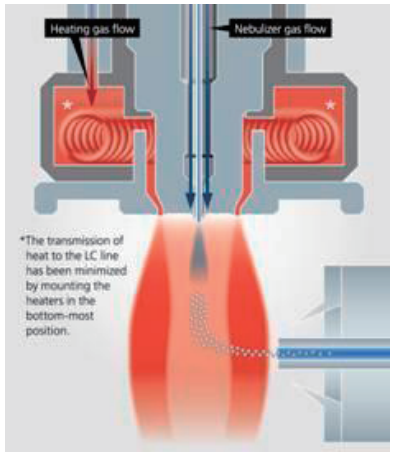

Figure 3. Heated ESI probe

LCMS-8045 triple quadrupole mass spectrometer by Shimadzu (shown in Figure 2), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe (shown in Figure 3) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.

LC/MS/MS analysis

Malachite green, leucomalachite green, crystal violet and leucocrystal violet were simultaneously analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8045 triple quadrupole system (Shimadzu Corporation, Japan). The details of analytical conditions are given in Table 1.

Table 1. Optimized LC/MS/MS conditions for dyes analysis

Column	: Shim-pack GISS (75mm L X 3.0mm I.D, 3 µm)
Mobile phase A	: 2 mM ammonium formate + 0.002 % formic acid in water.
	: B: 2 mM ammonium formate + 0.002 % formic acid in methanol
Flow rate	: 0.4 mL/min
Gradient program (B %)	: 0-0.5 min → 30 (%); 0.5-3 min → 30-95 (%); 3-4 min → 95 (%); 4-4.2 min → 30 (%); 4.2-6.5 min → 30 (%)
Injection volume	: 10 µL
Column temperature	: 40 °C
MS interface	: Electro Spray Ionization (ESI)
Nitrogen gas flow	: Nebulizing gas 3 L/min; Drying gas 10 L/min.
Zero air flow	: Heating gas 10 L/min.
MS temperature	: Desolvation line 150 °C; Heating block 400 °C; Interface 300 °C

Table 2. MRM transition of synthetic dyes

Sr.No.	Name of compound	Precursor m/z	Product 1 m/z	Product 2 m/z
1	Malachite green	329.25	313.15	208.10
2	Leucomalachite green	331.20	239.10	316.20
3	Crystal violet	372.35	356.20	340.15
4	Leucocrystal violet	374.30	358.25	239.15

Results

The LC/MS/MS method was developed for trace level quantitation of malachite green, leucomalachite green, crystal violet and leucocrystal violet in marine product. All MRM transitions were optimized with the help of auto optimization feature of LabSolutions.

Analysis was performed using aqueous as well as matrix matched standards. The MRM transition used for these analysis are given in Table 2. Linearity solutions were prepared from 0.05 to 0.5 ppb and acquired using external calibration method and result of linearity are tabulated in Table 3. Overlay of 0.05 ppb standards are shown in Figure 4. Matrix matched calibration levels were prepared and injected in the same concentration range. A control shrimp sample was extracted as per the same procedure. The Figure 5 shows overlay of chromatograms of blank, control sample and 0.05 ppb matrix matched standard clearly indicates that there no matrix interference. The calibration curve of for all four dyes are shown in Figure 6 and the correlation coefficient >0.99 was obtained for all compounds.

Highly sensitive and rapid analysis of synthetic dyes in sea food by LC/MS/MS

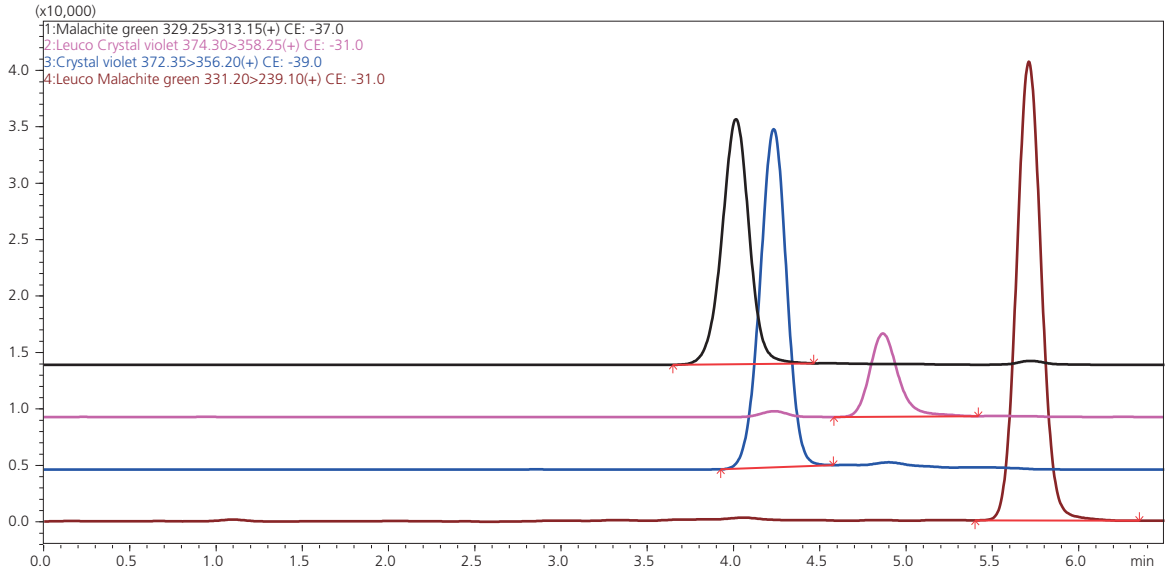

Figure 4. Chromatogram of solvent standard of 0.05 ppb

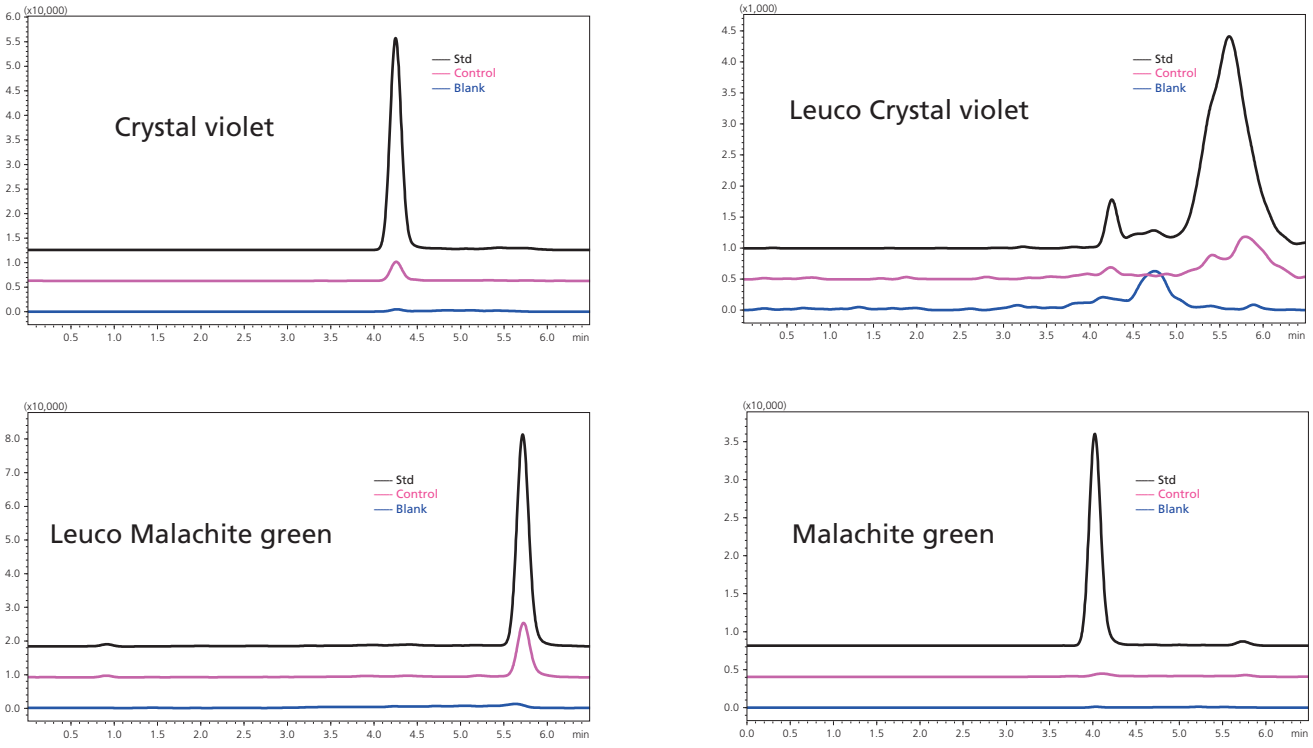

Figure 5. Overlay of chromatograms of blank, control and 0.05 ppb matrix standard

Highly sensitive and rapid analysis of synthetic dyes in sea food by LC/MS/MS

In earlier study it was observed that leuco malachite green and leuco crystal violet metabolite were not stable for long period of time in water or methanol even at -20 °C and oxidizes into non leuco form, hence aprotic solvent is used to prepare standards and at the same time ascorbic acid was used as an antioxidant.
SPE cartridge was used for extraction has strong cation exchange sorbent, since most of the dyes carry positive charge and due to this their retention was better which helps in reduction of matrix interference.

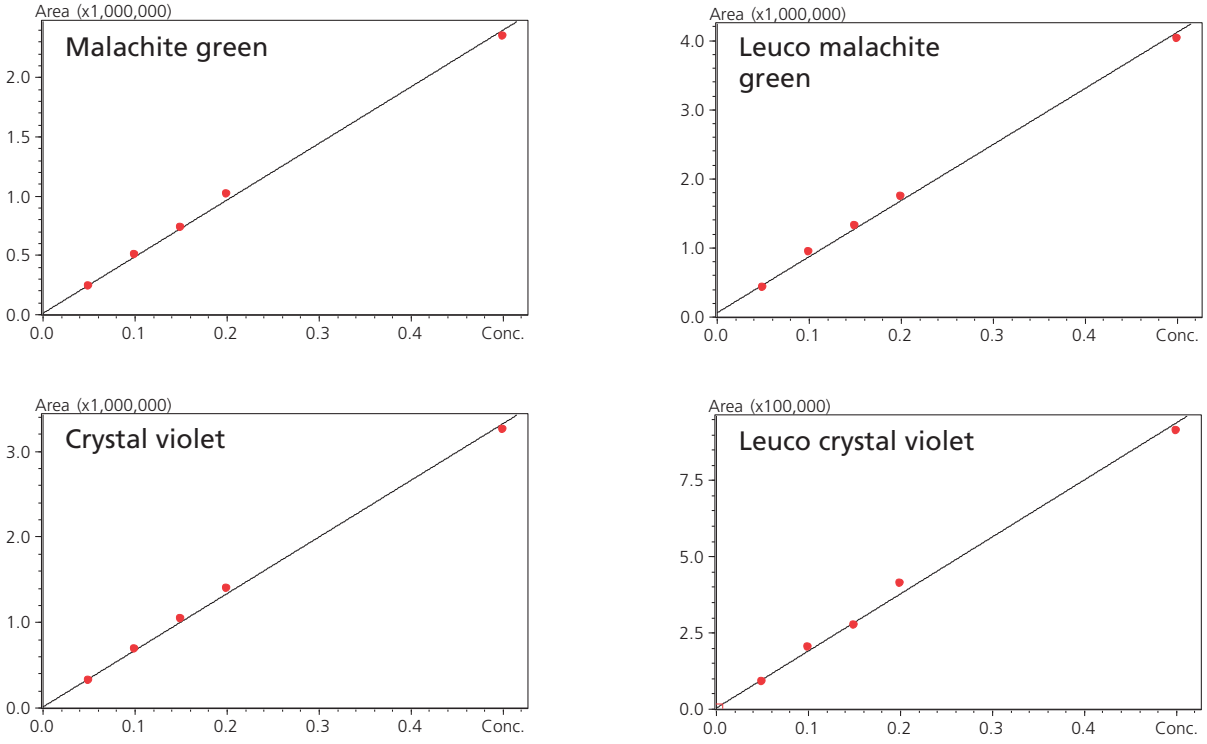

Figure 6. Calibration curves of dyes

Table 3. Linearity and recovery of dyes

Sr.No.	Name of compound	Linearity range (ppb)	Correlation coefficient (r^2)	% Recovery at 0.05 ppb
1	Malachite green	0.05-0.5	0.9974	80.21
2	Leucomalachite green	0.05-0.5	0.9960	112.00
3	Crystal violet	0.05-0.5	0.9977	95.00
4	Leucocrystal violet	0.05-0.5	0.9930	81.00

Highly sensitive and rapid analysis of synthetic dyes in sea food by LC/MS/MS

Conclusion

- A highly sensitive and rapid method for analysis of crystal violet, leuco crystal violet, malachite green and leuco malachite green was developed.
- Simple SPE method for the determination of malachite green, crystal violet and other synthetic dyes in seafood coupled with LC/MS/MS detection.
- Less matrix interference and sensitive LC/MS/MS instrument enabled lower detection limits and better recovery.

Reference

[1] A A Bergwerff, P Scherpenisse. J Chromatogr B: Anal Technol Biomed Life Sci. 788: 351-359
[2] Lopez-Gutierrez et al. Anal Methods. 5: 3434–3449, 2013.
[3] J C Hashimoto et al. J AOAC Int. 95: 913–922, 2012.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

First Edition: June, 2017

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/